

AMENDMENTS TO THE SPECIFICATION

Please replace paragraph [0041] with the following amended paragraph:

[0041] FIG. 5. Human Telomerase Reverse Transcriptase (hTERT) sequence (SEQ ID NO:23) [from Nakamura et al., 1997].

Please add the following new paragraph after paragraph [0042]:

[0042.1] FIG. 7. shows an exemplary diagram of the interaction between a cancer cell, T-cell and killer cells.

Please replace paragraph [0049] with the following amended paragraph:

[0049] hTERT synthetic peptides p540 (540ILAKFLHWL548) (SEQ ID NO:1), p865 (865RLVDDFLLV873) (SEQ ID NO:2) and MART-1 (27AAGIGILTV35) (SEQ ID NO:3) were purchased from the Biopolymer Synthesis Center (CalTech, Pasadena, Calif.). Synthetic peptides 128TPPAYRPPNAPIL140 (SEQ ID NO:4) of the hepatitis B core antigen (HBVc), 571YLSGANLNL579 (SEQ ID NO:5) of carcinoembryonic antigen (CEA), 476VLYRYGSFSV486 (SEQ ID NO:6) of melanoma antigen gp100, and 476ILKEPVHGV484 (SEQ ID NO:7) of HIV-1 reverse transcriptase were purchased from Neosystem (Strasburg, France).

Please replace paragraph [0057] with the following amended paragraph:

[0057] HHD mice were immunized subcutaneously at the base of the tail with 100 µg of individual hTERT peptide emulsified in incomplete Freund's adjuvant (IFA). Half of the mice were immunized with the hTERT peptide and 140 µg of the helper peptide TPPAYRPPNAPIL (SEQ ID NO:4), which corresponds to residues 128-140 of the hepatitis B core antigen (HBVc) (25).

Please replace paragraph [0059] with the following amended paragraph:

[0059] The relative avidity was measured as previously described (25). Briefly, T2 cells were incubated overnight at 37°C in RPMI supplemented with human β 2-microglobulin (100 ng/ml) (Sigma, St. Louis, Mo.) in the absence (negative control) or presence of the test peptide or the reference peptide 476ILKEPVHGV484 (SEQ ID NO:7) of HIV-1 reverse transcriptase at various final peptide concentrations (0.1-100 μ M). Cells were incubated with Brefeldin A (0.5 μ g/ml) for one hour and subsequently stained with a saturating concentration of monoclonal antibody BB7.2 for 30 minutes at +4°C followed by washing and a second incubation with a goat antibody to mouse Ig (Fab')₂ conjugated to FITC (Caltag, South San Francisco). Cells were then washed, fixed with 1% paraformaldehyde and analyzed in a FACs Calibur cytofluorimeter (Becton&Dickinson, San Jose, Calif.). The mean fluorescence intensity of each concentration minus that of cells without peptide was used as an estimate of peptide binding. Results are expressed as values of RA, which is the ratio of the concentration of test peptide necessary to reach 20% of the maximal binding by the reference peptide over that of the reference peptide so that the lower the value the stronger the binding. Dissociation of the test peptide from the HLA-A2.1 molecule reflects the half-life of fluorescence intensity of the peptide/MHC complex over time. The half-life of the complex (DC50) refers to the time (hours) required for a 50% reduction of the T0 mean fluorescence intensity (25). Synthetic peptides 571YLSGANLNL579 (SEQ ID NO:5) of carcinoembryonic antigen (CEA) and 476VLRYGSFSV486 (SEQ ID NO:6) of melanoma antigen gp100 were used as internal controls to account for inter-tests variability and for consistency with previously reported RA and DC50 measures (25).

Please replace paragraph [0063] with the following amended paragraph:

[0063] The amino acid sequence of hTRT (locus AF015950) (19) was analyzed for 9 mer peptide sequences containing known binding motifs for the HLA-A2.1 molecule [52; 35; 60], a subtype encompassing 95% of HLA-A2 allele which is expressed in about 50% of the Caucasian population (26-28). Peptides were identified by reverse genetics based on canonical anchor residues for HLA-A2.1 (29), and by using the software of the Bioinformatics & Molecular

Analysis Section (NIH) available at http://bimas.dort.nih.gov/molbio/hla_bind/index.html the corresponding website, which ranks 9 mer peptides on a predicted half-time dissociation coefficient from HLA Class I molecules (30). From an initial panel of ~30 candidate peptides Applicant retained two sequences, 540ILAKFLHWL548 (SEQ ID NO:1) and 865RLVDDFLLV873 (SEQ ID NO:2), denoted hereunder as p540 and p865.

Please replace paragraph [0064] with the following amended paragraph:

[0064] Since the immunogenicity of MHC Class I-restricted peptides reflects to some degree their binding and stabilizing capacity for MHC Class I molecules (31-33) Applicant sought direct proof of the strength of interaction between the two hTRT peptides and the HLA-A2.1 molecule in a conventional binding/stabilization assay that uses the antigen-transporting deficient (TAP-) HLA-A2.1+ human T2 cells. The relative avidity (RA) calculated in reference to 476ILKEPVHGV484 (SEQ ID NO:7) of HIV-1 reverse transcriptase, a canonical high binder peptide (25), was 2.9 and 2.5 for p540 and p865, respectively (Table I). The stability of each peptide bound to HLA-A2.1 was measured as the half-life of the complex

Please replace Table I (on page 17), with the following amended Table I:

TABLE I

Peptide origin/ designation	Sequence	Relative Avidity (RA) ^a	DC50 ^b
hTRT p540	ILAKFLHWL (<u>SEQ ID NO:1</u>)	2.9	4-6
hTRT p865	RLVDDFLLV (<u>SEQ ID NO:2</u>)	2.5	2-4
CEA p571 ^c	YLSGANLNL (<u>SEQ ID NO:5</u>)	3	>10
gp100 p476 ^d	VLYRYGSFSV (<u>SEQ ID NO:6</u>)	9	4-6

^aThe relative avidity of hTRT peptides was measured relative to the reference peptide ILKEPVHGV (SEQ ID NO:2) at a final peptide concentration of 0.1-100 μ M.

^bDC50 refers to the time required for a 50% reduction in mean fluorescence intensity.

^cPeptides of human carcinoembryonic antigen (CEA) (p571) and human melanoma antigen gp100 (p476) were used as internal controls for comparison with previously reported values³³.

Please replace paragraph [0070] with the following amended paragraph:

[0070] Cold target competition experiments were performed in an attempt to understand if lysis of the LnCap tumor cell line was specific for endogenously-processed hTRT peptides. In these experiments the lysis of LnCap cells by CTL from a prostate cancer patient was competed for by T2 cells pulsed in vitro with p540 or p865 (10 μ g/ml). Peptide-loaded T2 cells caused a dose-dependent inhibition of lysis of LnCap cells in both peptide combinations (FIG. 3,A). Applicant further assessed the specificity of the CTL generated against each one of the two hTRT peptides by testing them on T2 targets pulsed with irrelevant HLA-A2 binding peptides. Neither T2 cells pulsed with peptide 27AAGIGILTV35 (SEQ ID NO:3) from the melanoma antigen MART-1 nor T2 cells pulsed with a non-homologous hTRT peptide were lysed (FIG. 3,B). Collectively, these studies show that 1) patients' CTL are specific for the hTRT peptide used to induce them, and 2) lysis of prostate cancer cells is mediated by, and is specific for, endogenously-processed hTRT peptides complexed with HLA-A2.1 molecules, suggesting chemical identity between naturally processed peptides on tumor cells and the synthetic peptides used for immunization. Formal validation will require elution of peptides from tumor cells and their analysis by tandem mass spectrometry (35). Studies on MHC restriction were performed using blocking antibodies. Lysis of peptide-pulsed T2 cells by CTL lines generated from a prostate cancer patient was inhibited by the anti-MHC Class I monoclonal antibody BB7.2 in both peptide combinations (FIG. 3), but not by the anti-MHC Class II monoclonal antibody Q5/13 (36) nor by transfectoma antibody_IRGD3 that blocks NK cells (37). By two-color FACS analysis, the phenotype of T cells proliferating after three rounds of in vitro stimulation with hTRT peptide was CD3+ (78%), CD8+ (37%), CD4+ (36%) and CD16/56 (6%). Collectively, these experiments confirm that

effector T cells generated by in vitro immunization are MHC Class I-restricted (CD8+) T cells which do not possess NK activity.

Please replace Table II (on page 22), with the following amended Table II:

TABLE II
Induction of CTL Against hTRT in HLA-A2.1 Transgenic Mice

Group	hTRT Peptide	Helper Peptide	No. Responders	Percent Lysis
I	540ILAKFLHWL548 (<u>SEQ ID NO:1</u>)	-	10/15 (66%)	(35,21,34,42,56,21,12,35,42,16)
II	"	+	8/10 (80%)	(45,56,62,64,65,45,65,45)
III	865RLVDDFLLV873 (<u>SEQ ID NO:2</u>)	-	3/15 (20%)	(25,12,15)
IV	"	+	7/10 (70%)	(25,32,35,12,16,18,21)

^aHHD mice were immunized by a subcutaneous injection of 100 µg of hTRT peptide emulsified in incomplete Freund's adjuvant (IFA). In group 2 and 4 the hTRT peptide was administered together with 140 µg of the helper peptide TPPAYRPPNAPIL (SEQ ID NO:4)³³.

^bValues of cytotoxicity refer to individual responder mice. Spleen-derived CTL were harvested 7 days after immunization and then cultured for six days with the homologous hTRT peptide. Values refer to maximal cytotoxicity at an effector to target ratio of 60:1.

Please replace Table IV (on page 25), with the following amended Table IV:

TABLE IV
hTRT-Derived HLA-A2.1-Restricted Peptides

Anchor Position L at position 2 V at position 9	Anchor Position L at position 2 V at position 9	Anchor Position M at position 2 V, L or I at position 9
¹⁵² LLARCALFV ¹⁶⁰ (SEQ ID NO:8) ⁸⁶⁵ RLVDDFLLV ⁸⁷³ (SEQ ID NO:2)	⁹⁶ VLAFGFALL ¹⁰⁴ (SEQ ID NO:9) ⁶⁷⁵ LLGASVLGL ⁶⁸³ (SEQ ID NO:10) ⁷²⁴ RLTEVIASI ⁷³² (SEQ ID NO:11) ⁷⁹⁷ SLNEASSGL ⁸⁰⁵ (SEQ ID NO:12) ⁸³⁶ ILSTLLCSL ⁸⁴¹ (SEQ ID NO:13) ⁹²⁶ GLFPWCGLL ⁹³⁴ (SEQ ID NO:14) ¹⁰⁷² WLCHQAFLL ¹⁰⁸⁰ (SEQ ID NO:15) ⁵⁷² RLFFYRKS ⁵⁸⁰ (SEQ ID NO:16)	⁸¹² FMCHHAVRI ⁸²⁰ ⁸¹² FMCHHAVRI ⁸²⁰ (SEQ ID NO:17)

Please replace paragraph [0083] with the following amended paragraph:

[0083] Applicant used two such peptides 540ILAKFLHWL549 (SEQ ID NO:1) and 865RLVDDFLLV873 (SEQ ID NO:2), denoted as p540 and p865. Both peptides are able to induce a CTL response in vitro in normal blood donors and in patients with prostate cancer. Applicant has demonstrated that the same peptides are also able to induce a CTL response in vitro in patients with melanoma. A synopsis of these studies is shown in Table V.

Please replace Table VI (on page 27), with the following amended Table VI:

Table VI
Additional Sequence of Wild Type and Modified hTRT Peptides

<i>Name of Peptide</i>	<i>Wild Type Sequence</i>	<i>Modified Sequence</i>
p152	¹⁵² LLARCALFV ¹⁶⁰ (SEQ ID NO:8)	¹⁵² YLARCALFV ¹⁶⁰ (SEQ ID NO:18)
p555	⁵⁵⁵ ELLRSFFYV ⁵⁶³ (SEQ ID NO:19)	⁵⁵⁵ Y ELLRSFFYV ⁵⁶³ ⁵⁵⁵ YLLRSFFYV ⁵⁶³ (SEQ ID NO:20)
p572	⁷² RL FFYRKSV ⁵⁸⁰ ⁵⁷² RLFFYRKSV ⁵⁸⁰ (SEQ ID NO:21)	⁵⁷² YLFFYRKSV ⁵⁸⁰ (SEQ ID NO:22)